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(21) International Application Number: PCT/FI98/00667 (22) International Filing Date: 26 August 1998 (26.08.98) (30) Priority Data: 973501 26 August 1997 (26.08.97) FI 980119 20 January 1998 (20.01.98) FI (71) Applicant (for all designated States except US): CULTOR CORPORATION [FI/FI]; Kyllikinportti 2, FIN-00240 Helsinki (FI). (72) Inventors; and (75) Inventors/Applicants (for US only): ANTILA, Juhani [FI/FI]; Jääskentie 3 B, FIN-02140 Espoo (FI). RAVANKO, Vili [FI/FI]; Jämeräntäival 10 B 26, FIN-02150 Espoo (FI). WALLIANDER, Pertti [FI/FI]; Niittypolku 12, FIN-02460 Kantvik (FI). (74) Agent: KOLSTER OY AB; Iso Roobertinkatu 23, P.O. Box 148, FIN-00121 Helsinki (FI).		(81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: METHOD OF PREPARING L-ARABINOSE FROM SUGAR BEET PULP (57) Abstract The invention relates to a method of preparing crystalline L-arabinose by extraction of sugar beet pulp, from which sugar has been extracted, in a strong alkaline solution, by hydrolysis of the obtained crude araban with a strong acid at an elevated temperature, by neutralization and filtration of the obtained solution, by chromatographic separation of the L-arabinose fraction, by purification of the obtained L-arabinose solution by means of cation and anion exchangers and adsorbent resins, and by recovering the pure L-arabinose as a crystalline product.		

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METHOD OF PREPARING L-ARABINOSE FROM SUGAR BEET PULP

The invention relates to a method of preparing crystalline L-arabinose by extraction of sugar beet pulp, from which sugar has been extracted, in a strong alkaline solution, by hydrolysis of the obtained crude araban with a strong acid at an elevated temperature, by chromatographic separation of the L-arabinose fraction, by purification of the obtained L-arabinose solution by means of cation and anion exchangers and adsorbent resins, and by recovering the pure L-arabinose as a crystalline product.

DESCRIPTION OF THE PRIOR ART

L-arabinose has been prepared by acid hydrolysis from arabinose-containing vegetable materials, such as gum arabic. Another well known raw material is sugar beet pulp from which sugar has been extracted; L-arabinose has been prepared from this material by alkaline or acid hydrolysis followed by multistep purification. US Patent 4,816,078, for example, teaches a method of preparing L-arabinose from sugar beet pulp by hydrolysis in the presence of lime, by filtration and chromatographic separation of the araban fraction; whereupon the araban is subjected to hydrolysis by addition of acid, and the obtained L-arabinose is separated by chromatography using a cation exchanger in Ca-form. According to a previous method described in GB published application 1,182,099, L-arabinose is prepared from a beet pulp hydrolyzate by crystallization from an alcoholic solution.

BRIEF DESCRIPTION OF THE INVENTION

It has now been discovered that L-arabinose can be obtained with good yield from sugar beet pulp, from which sugar has been extracted, by simple alkaline extraction, by acid hydrolysis and chromatographic separation using as separating resin a cation exchanger in monovalent metal (Na^+) form, to obtain an L-arabinose-containing fraction. As a last step, the L-arabinose is crystallized from an aqueous solution. The method avoids the previous multiple separation and purification steps.

DETAILED DESCRIPTION OF THE INVENTION

The method of the invention enables the preparation of pure L-arabinose. In accordance with the invention, sugar beet pulp, from which sugar has been extracted, is extracted with an alkaline solution to dissolve the araban. After filtration the alkaline extract is hydrolyzed with acid to obtain a

crude L-arabinose solution. The crude arabinose fraction is subjected to chromatographic separation by using a cation exchanger resin in monovalent metal (Na^+) form (e.g. sulphonated polystyrenedivinyl benzene; 5.5% DVB) and water as eluent. The solution is then purified by ion exchange. The solution is concentrated to about 70 percent by weight, and the pure L-arabinose is crystallized. The crystals are separated by centrifugation and dried in air.

The separating resin used in the chromatographic separation is preferably sulphonated polystyrenedivinyl benzene (5.5% DVB) in monovalent metal (Na^+ , K^+) form. Purification and colour removal is carried out with cation and anion exchange resins and other adsorption resins.

As feedstock is preferably used sugar beet pulp stabilized by the biotechnical method according to Finnish Patent Application 973,501. The following presents a general flow chart.

15 **Sugar beet pulp**
Aqueous suspension containing about 5 to 10% dry substance

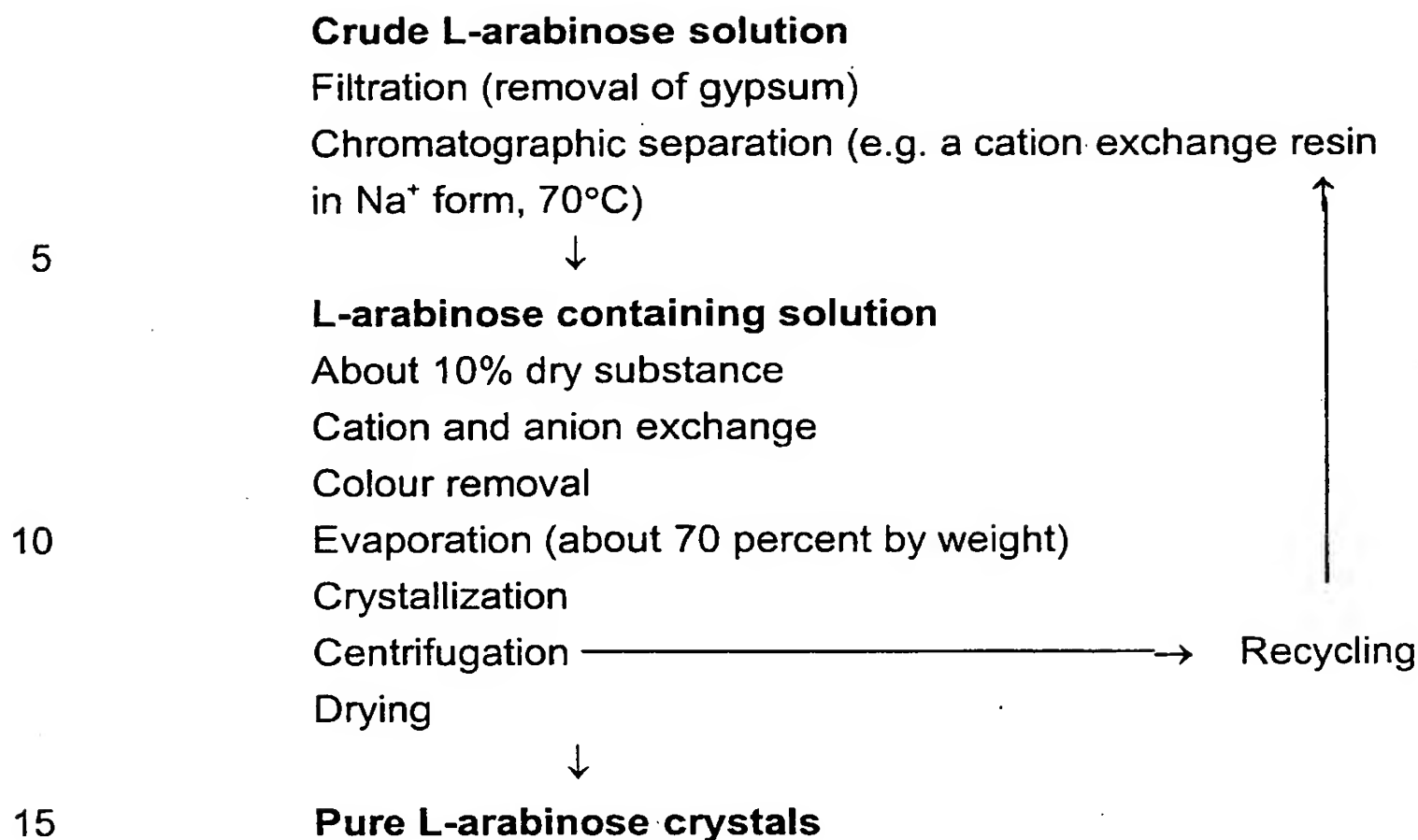
Alkaline extraction (e.g. $\text{Ca}(\text{OH})_2$ suspension, pH 10 to 12,
95 to 100°C, 4 to 5 h)
20 Neutralization with acid (pH below about 9)
Filtration



25 **Crude araban solution**
Evaporation (40 to 50 percent by weight)
Hydrolysis (e.g. H_2SO_4 , 90°C, pH 0.8)
Neutralization (e.g. NaOH , pH about 6)

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PREFERRED EMBODIMENT OF THE INVENTION

In accordance with a preferred embodiment of the invention, sugar beet pulp, from which sugar has been extracted, is suspended in a strong alkaline solution and extracted for 4 to 5 hours at a pH of about 10 to 12 and a temperature of 95 to 100°C. The alkaline "solution" is preferably milk of lime containing 25 to 40 percent by weight of Ca(OH)₂ calculated on the dry substance of the sugar beet pulp. Optionally, buffered alkaline solutions (KOH, NaOH) can be used in the extraction. The obtained mixture is neutralized with e.g. carbon dioxide or optionally sulphuric acid to about pH 9 or below to precipitate the calcium salt. After filtration the solution is concentrated to about 40 percent by weight and then subjected to acid hydrolysis, e.g. by addition of sulphuric acid, to pH about 0.8. The hydrolysis is carried out at about 90°C, whereupon the solution is cooled to about 70°C and neutralized by addition of an alkali (e.g. NaOH) to pH about 6. The neutralized solution is subjected to chromatographic separation by using a cation exchange resin in monovalent metal form, preferably Na⁺ form. The separated arabinose-containing fraction has a dry substance content of 5 to 10%, of which 75 to 80% is L-arabinose. Pure L-arabinose crystals are obtained by evaporation (a solution of about 70 percent by weight), seeding and cooling crystallization. The crystals can be

separated by centrifugation.

Another preferable way to extract and hydrolyze araban is to continue by acid hydrolysis after alkaline hydrolysis to degrade the araban into L-arabinose. In this embodiment the alkaline extraction takes about 4 to 5 hours, whereupon the pH is adjusted to about 0.8 with sulphuric acid. The extract is treated at 90°C until the araban is degraded, whereupon the suspension is neutralized (pH 6), filtered by a filter press and concentrated by evaporation for chromatographic separation.

The choice of embodiment depends on equipment capacity and the suitability of the raw material.

EXAMPLE 1

Calcium hydroxide in an amount of 30 to 40 percent by weight was added to hot water (95 to 100°C). Dried sugar beet pulp, from which sugar had been extracted and which had a coarseness of about 0.5 cm, was suspended in the obtained milk of lime to a solution of about 8 percent by weight, and mixed for 3 to 4 hours at a temperature of 95 to 100°C and a pH of 10 to 12. The mixture was cooled to 75°C and neutralized by addition of carbon dioxide to pH 9 to precipitate the calcium carbonate. The neutralized mixture was filtered in a filter press.

The filtrate which contained 4% of polysaccharides (crude arabino-galactan) was concentrated to 40 percent by weight by evaporation and sulphuric acid was added to pH 0.8. The araban was hydrolyzed at 90°C, whereupon the solution was cooled to 70°C and 10 percent by weight sodium hydroxide solution was added to pH 6. The neutralized solution was filtered and the L-arabinose separated by chromatography by using a sulphonated polystyrenedivinyl benzene cation exchange resin in Na⁺ form. An L-arabinose fraction containing 8% of dry substance (of which 80% was L-arabinose) was recovered. The L-arabinose solution was concentrated to 30 percent by weight by evaporation and purified by cation and anion exchange followed by colour removal with Optipore® (Dovex®) adsorption resin (manufacturer Dow Chemicals, USA). The purified solution was concentrated to 70 percent by weight, seeded with arabinose crystals and allowed to crystallize by cooling to room temperature. The crystals (arabinose purity over 98%) were separated by centrifugation and dried in a hot air current. The yield of L-arabinose crystals was 10 percent by weight of the pulp dry substance.

EXAMPLE 2

Fresh sugar beet pulp, from which sugar had been extracted and which had a coarseness of about 0.5 cm and which had been pre-treated with biotechnical pre-treatment (described in Patent Application 973,501 and in Example 3), was suspended into hot (95 to 100°C) milk of lime (30 to 40% Ca(OH)_2 of the beet pulp dry substance) to a mixture of 7 percent by weight. The mixture was extracted as in Example 1, the extracted pulp was separated from the solution by a decanting centrifuge and the crude araban was hydrolyzed as in Example 1. The L-arabinose was separated by chromatography and purified by ion exchange and colour was removed as in Example 1. The L-arabinose was crystallized from the concentrated solution with a yield of 15% of the pulp dry substance.

EXAMPLE 3

Fresh pressed almost sugarless sugar beet pulp (dry substance 22%) was mixed in an "Ensimax" (a mixture of formic acid, acetic acid and liginosulphonate, manufacturer Kemira Oy, Finland) acid mixture to pH 4. The mixture was packaged in a tight container which protects the contents from air and light. The mixture was allowed to stabilize for a month. The pre-treatment removes oxygen and free sugars from the mixture and the treated pulp is consequently stable for at least a year when stored at a non-elevated temperature. The pre-treated pulp is used as raw material in L-arabinose production.

EXAMPLE 4

The crude arabinose solution obtained from acid hydrolysis was filtered and subjected to chromatographic separation to purify L-arabinose from other components.

The separation was performed in a pilot chromatographic column as a batch process. A cation exchange resin (manufacturer Finex Oy, Finland) in Na^+ form was used. The resin was sulphonated polystyrene which was cross-linked with divinyl benzene; the cross-linkage degree of the resin was 5.5% and average particle size 0.35 mm. The inner diameter of the column was 1.0 m and the height of the resin bed was about 5.2 m, resulting in a resin volume of about 4,000 litres. The separation was carried out at 80°C (a heated column was used) with ion exchanged water as eluent.

The separation process was carried out as follows:

Step 1:

About 400 litres of arabinose solution (125 kg dry substance) was applied to the column through a heat exchanger using a flow rate of about 750
5 l/h. The dry substance content of the solution was about 35 g dry substance in 100 g solution.

Step 2:

The eluent (ion exchanged water) was led downwards in the column
10 using a flow rate of 650 l/h. The eluent was also applied through the heat exchanger.

Step 3:

The density and conductivity of the solution discharged from the
15 column was measured and recorded continuously. According to this information, the solution was divided into two fractions: a residual fraction (containing salts and small amounts of glucose, galactose and fructose) and an arabinose fraction (containing arabinose and small amounts of galactose and fructose).

A recycle fraction containing glucose, galactose, fructose and a
20 small amount of arabinose can also be recovered between the two fractions. The recycle fraction can be reapplied into the column or used for diluting the feed solution.

The amounts of dry substance (DS) and the compositions of the arabinose fraction and the feed solution are presented in Table 1. The concentrations of the components are expressed as percent by weight of the total
25 dry substance of the fraction. The yield expresses the amount of L-arabinose in the arabinose fraction in relation to the total amount of L-arabinose calculated on the basis of the amounts of L-arabinose in both obtained fractions.

Table 1. Values and compositions of feed solution and arabinose fraction, and L-arabinose yield

	Feed solution	Arabinose fraction
DS in fraction, kg	127.8	39.2
DS, g/100 g solution	35.5	9.8
Conductivity, mS/cm	30.9	1.4
pH	5.1	4.7
Glucose, % of DS	1.8	0.7
Galactose, % of DS	1.3	2.1
Fructose, % of DS	1.2	2.9
Arabinose, % of DS	24.7	77.6
Arabinose yield, %		96.3

Arabinose yield is the relation of the amount of arabinose in the arabinose fraction to the amount of arabinose in the feed solution (%).

EXAMPLE 5

Crude arabinose solution obtained from acid hydrolysis was filtered with a pressure filter and fractionated by chromatography in plant scale. The separating column employed was a conventional batch separation column, height 7.0 m and inner diameter 2.8 m.

The separation resin used was a cation exchange resin in Na⁺ form (manufacturer Finex Oy, Finland). The resin was sulphonated polystyrene cross-linked with divinyl benzene; cross-linkage degree was 5.5% and particle size about 0.45 mm. The height of the resin bed was about 6.4 m and the resin volume was consequently about 38 m³. The temperature in the column and the feed solution tank was about 70°C.

The principle of the separation is the same as in Example 1 (steps 1 to 3). Feed size was about 2,000 litres (780 kg dry substance), i.e. the concentration was 34 g in 100 g of solution. Flow rate in the column was about 5 m³/h. Two fractions were recovered as in Example 1. Table 2 shows the dry substance (DS) contents and compositions of the feed solution and the arabinose fraction.

Table 2. Values and compositions of feed solution and arabinose fraction, and L-arabinose yield

	Feed solution	Arabinose fraction
DS in fraction, kg	777	157
DS, g/100 g solution	33.9	5.6
pH	~6	~5
Glucose, % of DS	1.7	0.5
Galactose, % of DS	2.4	3.5
Fructose, % of DS	1.2	3.1
Arabinose, % of DS	18.0	80.5
Arabinose yield, %		90.4

The yield was calculated as in the previous example.

5 EXAMPLE 6

Calcium hydroxide in an amount of 40 percent by weight calculated on the dry substance of the beet pulp to be treated was added to hot water at about 98°C. The dry, almost sugarless pulp was suspended in an alkaline solution to a mixture of about 9 percent by weight, and the mixture was mixed for 4 hours at a temperature of 98°C and a pH of about 10. Sulphuric acid was then added to the mixture to pH 0.8 and the araban was hydrolyzed into L-arabinose at a temperature of 90°C. The suspension was cooled to 70°C and 10 percent by weight sodium hydroxide solution was added to pH 6.0. The neutralized mixture was filtered by a filter press and concentrated to 40 percent by weight.

The solution was fine-filtered by a Seitz filter by means of siliceous earth, whereupon the L-arabinose was separated from the solution by chromatography as in the previous examples. An L-arabinose fraction having a dry substance content of 8 percent by weight, of which 80% was L-arabinose, was recovered. The solution was concentrated by evaporation to 30 percent by weight, purified by cation and anion exchange and by colour removal resin (Optipore®/Dovex®), and evaporated to 70 percent by weight. The solution was then seeded and the L-arabinose crystallized by cooling the solution to room temperature. The purity of the crystals exceeded 98%. The arabinose yield was 10% calculated on the dry substance of the raw material.

CLAIMS

1. A method of preparing crystalline L-arabinose, **characterized** by the combination of the following steps:
 - a) extraction of sugar beet pulp, from which sugar has been extracted, in a strong alkaline solution,
 - b) hydrolysis of the obtained crude araban with a strong acid at an elevated temperature,
 - c) neutralization and filtration of the obtained solution,
 - d) chromatographic separation of the L-arabinose fraction by using a cation exchanger in monovalent metal form as separation resin,
 - e) purification of the obtained L-arabinose solution by means of cation and anion exchangers and adsorbent resins, and
 - f) recovery of the pure L-arabinose as a crystalline product.
2. A method as claimed in claim 1, **characterized** in that in step a) the extraction is carried out at pH 10 to 12.
3. A method as claimed in claim 2, **characterized** in that a calcium hydroxide suspension is used as the alkali.
4. A method as claimed in claim 1, **characterized** in that in step c) pH is 6 at the end of neutralization.
5. A method as claimed in claim 4, **characterized** in that neutralization is carried out with carbon dioxide or sulphuric acid.
6. A method as claimed in claim 1, **characterized** in that in step b) the hydrolysis is carried out at a pH of about 0.8 at a temperature of about 90°C.
7. A method as claimed in claim 1, **characterized** in that in step d) the cation exchanger is in Na⁺ form.
8. A method as claimed in claim 1, **characterized** in that as feedstock is used sugar beet pulp stabilized by a biotechnical method.
9. A method as claimed in claim 8, **characterized** in that the pH of the stabilized sugar beet pulp is 3.5 to 4.5 (preferably 3.9 to 4.3) and the dry substance content 20 to 27 percent by weight, and the pulp is substantially free from fermentable sugars.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 98/00667

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C13K 13/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07H, A23L, C13K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPODOC, WPI, CAPLUS, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4816078 A (HUBERT SCHIWECK ET AL), 28 March 1989 (28.03.89) --	1-9
A	DD 143261 B (AKADEMIE DER WISSENSCHAFTEN DER DDR), 13 August 1980 (13.08.80) -- -----	1-9

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

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INTERNATIONAL SEARCH REPORT

Information on patent family members

03/11/98

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4816078 A	28/03/89	DE 3702653 A,C EP 0276702 A,B SE 0276702 T3	11/08/88 03/08/88
DD 143261 B	13/08/80	NONE	

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